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## A NOTE ON THE ISOLATION OF VIRULENT DIPHTHERIA BACILLI FROM WOUNDS OF HORSES.

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*From the Wellcome Physiological Research Laboratories, Beckenham, Kent.*



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IN a recent paper, our colleague, A. T. Glenny (1925), has shown that horses possessing natural diphtheria antitoxin in their blood are the most suitable for artificial immunization. Although large numbers of animals contain this natural antibody, its origin has never been satisfactorily traced. That it is due to the specific stimulus of infection by the toxigenic diphtheria bacillus is the most likely explanation, which seems to be borne out by the sudden increases in antitoxic titre, which tend to occur amongst naturally immune horses after arrival at a large stud and before they have received injections of diphtheria toxin. However, recorded observations on the occurrence of virulent *C. diphtheriae* in horses are not numerous, although much work has been done in this connection.

Cobbett (1900) recorded a case where a true diphtheria bacillus was isolated from a sick pony, which developed a purulent and blood-stained nasal discharge associated with laryngitis and nasal obstruction. Shortly before this illness was observed its rider had fallen ill with diphtheria. The bacillus recovered from the nasal mucosa was morphologically indistinguishable from *C. diphtheriae*, and fermented glucose. It was toxigenic and pathogenic to guinea-pigs, producing the typical post-mortem appearances of diphtherial infection.

Minett (1920) published an account of his bacteriological investigations on samples of pus derived chiefly from suspected cases of ulcerative lymphangitis. He was able to isolate diphtheria bacilli from the lesions of eleven horses and one mule, and showed that five of these strains yielded demonstrable toxin. Six of Minett's cultures were re-examined by Petrie (1921), who reported that they were indistinguishable from true diphtheria bacilli in all cultural, serological and toxigenic properties.

Kliewe and Westhues (1925) claimed to have isolated 12 strains of virulent diphtheria bacilli from the wounds of 70 horses which they examined. In the majority of these cases the tests of virulence were not convincing, and only in two do they appear to have been submitted to an antitoxin control.

On several occasions we had examined the nose and throat of a considerable number of horses and their attendants for the presence of *C. diphtheriae*, but had failed to find a positive culture. Last summer, however, at Mr. Glenny's suggestion we examined thirteen swabs taken by our colleague, Mr. Dalling, from various wounds of horses at a veterinary hospital in Forfarshire. None of these wounds showed membrane or other features suggesting the presence of diphtheria bacilli. Diphtheria bacilli were isolated in three instances, and were shown to be virulent by the intracutaneous and subcutaneous methods

of test. Guinea-pigs injected intraperitoneally with antitoxin survived, but unprotected animals died with all the typical post-mortem appearances of diphtheria.

The fermentative reactions were those of *C. diphtheriae*, acid being formed from glucose, maltose, galactose, dextrin and glycerol, but not from sucrose, lactose, dulcite, mannite, salicin, raffinose and litmus milk.

Each of the strains yielded a filtrate of which 0.1 c.c. killed a 250 gm. unprotected guinea-pig in 48 hours when injected subcutaneously, but not a control animal protected by the intraperitoneal injection overnight of 500 units of diphtheria antitoxic serum. The presence of toxin in the filtrate was also confirmed "in vitro" by the Ramon flocculation method.

#### SUMMARY.

1. We have never obtained virulent *C. diphtheriae* from the throat or nose of numerous normal horses examined at various times.

2. Typical and fully virulent *C. diphtheriae* have been isolated from three out of thirteen swabs taken from wounds of horses at a veterinary camp.

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